Cell extraction with autolysis combined with bead-milling

This Method is used for cell extraction via autolysis and subsequent cell wall disruption with bead-milling.

Sample preparation

For autolysis, *C. vulgaris* and *C. sorokiniana* were prepared as shown in the following table, baker's yeast served as a control.

Table 1: List of samples and their pH for autolysis

Sample	Sample ID	pH-value	cell dry weight [mg]
baker's yeast	BY1	3	300
baker's yeast	BY2	3	300
baker's yeast	BY3	12	300
baker's yeast	BY4	12	300
C. vulgaris	CV1	3	300
C. vulgaris	CV2	3	300
C. vulgaris	CV3	12	300
C. vulgaris	CV4	12	300
C. sorokiniana	CS1	3	300
C. sorokiniana	CS2	3	300
C. sorokiniana	CS3	12	300
C. sorokiniana	CS4	12	300

For each organism two samples were used (double determination). To have the same amount of biomass in each sample cell dry weight was calculated form OD-CDW correlations. For chlorella vulgaris we used our own OD-CDW correlation from dilution. For baker's yeast a correlation from A. Kusterer [1] was used. For *C. sorokiniana* we used the correlation form C.Y. Lim [2].

Autolysis protocol

1. The samples were centrifuged at 3000 rpm for 20 minutes. Supernatant was discarded.

2. Samples were resuspended in DSN medium.

3. pH-value of the samples was set to pH 3 with HCl or pH 12 with NaOH with a pH-Meter (digital pH-Meter wtw)

4. Samples were heated to 50 °C for 41 h and shook at 120 rpm (AquaShaker Kühner AG Schweiz)

5. Samples were neutralized with NaOH or HCl, respectively.

Cell Disruption

1. 3 mL of every sample was used for bead-milling.

2. Prepared tubes with beads from “quick DNA fungal/bacterial Mini-Prep Kit” by Zymo were used.

3. Samples were bead-milled with “retsch RKM Typ NN2” at maximum speed for 20 minutes.

4. To get rid of the beads, filters from “quick DNA fungal/bacterial Mini-Prep Kit” by Zymo were used. The samples were put on the filter and centrifuged at 11,000 rpm for 5 minutes. The flow through was kept for further analyses with the rFAN-Assay. The Assay was performed as described in the according protocol.

Literature

[1] A. Kusterer, „Reaktionstechnische Optimierung von Parallelreaktoren für kontrollierte Bioprozesse“, Dissertation, TUM Munich, 2007.

[2] Lim, et al. „A strategy for urban outdoor production of high-concentration algal biomass for green biorefining”, Bioresource Technology, v.135, 2013, pp. 175-181.